# A COMPARISON

of

THE ANTIGENIC VALUES

of the various

COMPONENT FRACTIONS

of

BACILLUS TYPHOSUS

(as obtained by the chemical processes used in Thomson's Method of "detoxication",)

"DETOXICATED TYPHOID VACCINE"

(Thomson's - as marketed)

with

THE ANTIGENIC VALUES

of

"WHOLE BACILLUS TYPHOSUS VACCINE"



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The object of the following investigation is to ascertain if the detoxication of organisms is detrimental to their antigenic properties and to ascertain if any immunity is produced and if so which fraction or fractions are responsible for it.

As Bacillus Typhosus is not a difficult organism to grow in large quantities, it was chosen to experiment with. The ordinary laboratory strain of Bacillus Typhosus was grown on Trip Agar until a semisolid mass of about 3 cc was collected. This was detoxicated according to Thomson's method[1] (summarised here) and the component parts were obtained in two stages as follows.

STAGE 1.

A. Isolation of Alkali Soluble Substance.

The tube containing the semisolid mass 5 cc of bacteria, 10 cc N/1 NaOH were added: the mixture was stirred well and put into the 37. 5° C incubator for six hours by which time the germs had dissolved into a semi-transparent fluid. This was filtered through glazed filter paper. The filtrate represented the alkali soluble portion of the bacillus.

B. Isolation of the Acid Soluble Substance.

The alkali insoluble material was then washed off the filter paper with N/1 HCl into another tube and after thorough mixing with the acid was allowed to stand in the incubator for a few hours, after which the mixture was filtered through glazed filter paper. The second filtrate represented the acid soluble fraction.

C. Isolation of the Alcohol Soluble Substance.

The material left on the filter paper was washed off with absolute alcohol and after standing in the incubator for a few hours was filtered. This third filtrate represented the alcohol soluble fraction.

D. Isolation of the Chloroform Soluble substance.

The material left on the filter paper was washed off with chloroform; placed in the incubator as before and again filtered. This fourth filtrate contained the chloroform soluble fraction.

STAGE 2.

The second stage in the process of detoxication consisted in precipitating each fraction from its respective solution.

1. Precipitation of the alkali soluble fraction.

To the tube containing the alkali soluble material $^{N}/_{1}$ HCl was added until the maximum amount of precipitation had occurred. (This precipitate was soluble in excess HCl). The tube was then centrifuged at high speed, the deposit was kept and the supernatant fluid which contained the proteoses was poured into another tube for further treatment.

2. Precipitation of the Acid Soluble Fraction.

To the tube containing the acid soluble material $^{N}/_{1}$ NaOH was added until a light flocculent precipitate was produced. The deposit was then separated from the supernatant fluid by centrifuging and both components were kept for further treatment.

3. Precipitation of the Alcohol soluble fraction.

To the tube containing the alcohol soluble portion of the germ twice the volume of normal saline was added; a very fine precipitate was produced which flocculated into larger masses on standing for two days. When this occurred it was filtered and the precipitate was washed off the filter paper with sterile saline to which .5% carbolic acid was added.

4. Precipitation of the chloroform soluble fraction.

The tube containing this fraction was placed in a drying oven until the bulk was considerably reduced by evaporation, then excess of absolute alcohol was added. No deposit was found.

Thomson found no chloroform soluble fraction in Bacillus Typhosus. (2).

5. Precipitation of the proteoses.

The supernatant fluids obtained after precipitation of the alkali soluble and acid soluble fractions were kept as mentioned under (1) and (2). These fluids were brought to a neutral reaction; twice their volume of absolute alcohol was added; and they were then allowed to stand until the proteoses separated out (about 12 hours). They were driven down in the centrifuge.

The precipitates obtained were washed with saline. Thomson advised washing with absolute alcohol but later he discarded this treatment because he found by experiment that alcohol tended to reduce the antigenic properties of the fractions (3).

The proportions of these fractions obtained; without actual measurement appeared to confirm the proportions found by Thomson (4).

| | | |
|---|---|---|
| i.e. | Alkali soluble | 60% |
| | Acid Soluble | 27% |
| | Alcohol soluble | 2% |
| | Chloroform soluble | 0% |
| | Proteoses | 11% |

The immune sera were obtained in the following manner.

A 24 hours growth on an agar slope was suspended in 10 cc saline and sterilised for ½ hour at 60° C. This was used to immunise the positive control rabbit. The fractions obtained were also suspended in 10 cc's saline and the rabbits were immunised with doses which roughly correspond to one agar slope. About 100 agar slopes of Bacillus Typhosus gave 1 cc of a semisolid mass after centrifuging for one hour at 3,000 revolutions.

Allowance was made for at least a 20% loss on the total amounts of the fractions during the process of detoxication (5) i.e. .1 of an agar slope corresponded to 1. 25 cc of a $\frac{1}{500}$ dilution of suspension of alkali soluble fraction obtained. The innoculations were subcutaneous and were made as follows :-

| | Rabbit 1. | Rabbit 2. | Rabbit 3 | Rabbit 4. | Rabbit 5. |
|---|---|---|---|---|---|
| | WITH B TYPHOSUS. | WITH ALKALI SOLUBLE FRACTION | WITH ACID SOLUBLE FRACTION | WITH ALCOHOL SOLUBLE FRACTION | WITH PROTEOSES. |
| 1st DAY | .1 of an Agar Slope of B TYPHOSUS | 1·25cc of 1/500 dilution | 1·25cc of 1/500 dil^n | 1·25cc of 1/500 dil^n | 1·25cc of 1/500 dilution |
| 4th " | .1 " | 1·25 cc " " | 1·25cc " " | 1·25cc of " | 1·25 cc " " |
| 13th " | .2 " | 2·5 cc " | 2·5cc " " | 2·50 cc " " | 2·5cc " " |
| 19th " | .2 " | 2·5 cc " " | 2·5 cc " " | 2·5 cc " " | 2·5 cc " " |
| 27th " | .4 " | 1·0cc 1/100 dilution. | 1·0 cc of 1/100 dilution | 1·0 cc 1/100 dilution | 1·0 cc 1/100 dilution. |

Only rabbit 1. showed any reaction clinically ( it did not move about in its cage and refused food ). With the subsequent innoculations the reactions diminished and after the last one on the 27th the rabbit showed no obvious symptoms. The other rabbits had no reaction to their innoculations.

The sera were drawn off on the 37th day and tested for the following qualities :-

1. presence of agglutinins (by Dreyer's technique)
2. fixation of complement (by a modified No. 4 method) and the results were as follows :-

RESULTS OF AGGLUTINATION TESTS

RABBIT II

| SUSPENSION USED | IMMUNE TYPHOID SERUM 1/25 1/50 1/100 1/1000 Master | IMM. ALKALI SOL SERUM. 1/25 1/100 1/1000 | IMM. ACID SOL. SERUM 1/25 1/100 1/1000 | IMM ALCOHOL SOL SERUM 1/25 1/100 1/1000 | IMM PROTEOSES. SERUM. 1/25 1/100 1/1000 | NORMAL SERUM 1/25 1/100 1/1000 | IMM ALK. SOL. SERUM 1/25 1/100 1/1000. |
|---|---|---|---|---|---|---|---|
| "WHOLE" TYPHOID VACCINE | + + + + + | — — — | — — — | — — — | — — — | — — — | — — — |
| DREYERS STANDARD EMULSION | + + + + + | — — — | — — — | — — — | — — — | — — — | — — — |
| ALKALI SOLUBLE SUBS. | PRECIPITATION. NO TRUE AGGLUTINATION ↓ ↓ ↓ — — | — — — | — — — | — — — | — — — | — — — | — — — |
| ACID SOL. SUBS. | ↓ ↓ — — — | — — — | — — — | — — — | — — — | — — — | — — — |
| ALCOHOL SOL. SUBS. | ↓ — — — — | — — — | — — — | — — — | — — — | — — — | — — — |
| PROTEOSES | ↓ — — — — | — — — | — — — | — — — | — — — | — — — | — — — |

RESULTS OF COMPLEMENT FIXATION TESTS.

| ANTIGEN USED | IMM. TYPHOID SERUM COMPLEMENT 2 doses | 4 doses | IMM ALK SOL SERUM 2 doses | 4 doses | IMM ACID SOL SERUM 2 doses | 4 doses | IMM ALCOHOL SOL SERUM 2 doses | 4 doses | IMM PROTEOSES SERUM 2 doses | 4 doses | NORMAL SERUM 2 doses | 4 doses | IMM ALK SOL SERUM 2 doses | 4 doses |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| "WHOLE" TYPHOID VACCINE | + | + | — | — | — | — | — | — | — | — | — | — | — | — |
| ALKALI SOL. SUBS | + | + | — | — | — | — | — | — | — | — | — | — | — | — |
| ACID " " | + | + | — | — | — | — | — | — | — | — | — | — | — | — |
| ALCOHOL " " | + | — | — | — | — | — | — | — | — | — | — | — | — | — |
| PROTEOSES " " | + | ± | — | — | — | — | — | — | — | — | — | — | — | — |

The antigens were prepared (1) from one agar slope of Bacillis Typhosus suspended in saline about 1,000 millions per cc and the "fraction" antigens were the materials left over from the immunisation of the rabbits. With the exception of the serum from the rabbit immunised against "whole" Bacillus Typhosus, no other serum had power to agglutinate Bacillus Typhosus or to fix complement in the presence of a suspension of Bacillus Typhosus as antigen or to fix complement in the presence of their homologous antigens. The "whole" typhoid immune serum agglutinated a suspension of Bacillus Typhosus and Dreyer's standard suspension

up to 1 in 10,000 and caused a precipitation of the fractions in low dilutions only. It also fixed complement with the fraction suspensions as antigens. With the alsohol soluble substance only two doses were fixed and with the proteoses fraction two but not quite four doses were fixed.

Thomson states that the amount of antibody obtained depends on the amount of antigen innoculated[6]. As it was considered that the immunising doses were too small the experiments were repeated.

A fresh supply of Bacillus Typhosus was grown on agar medium on large petri dishes each giving a surface of about a square foot. The Bacilli were grown as quickly as circumstances permitted, so that as few antigenic properties as possible would be lost by keeping until a sufficient quantity was collected. The Bacilli were centrifuged for one hour at 3,000 revolutions per minute and a semisolid mass of 10 cc was obtained. This mass was submitted as before to Thomson's method of detoxication. The fractions so obtained were suspended in 12 cc normal saline. Each suspension was divided into two equal portions, one of which was used to immunise a rabbit; the other was kept for use as an antigen. A fresh series of rabbits was obtained.

Of these one rabbit was kept as a negative control; one was immunised with ordinary "whole" typhoid vaccine 1,000 millions per cc; and a third was immunised with "detoxicated" typhoid vaccine 20,000 millions per cc (Genatosan) - The rabbit immunised with detoxicated typhoid vaccine received 28½ times the dose of one immunised with "whole" typhoid vaccine.

The innoculations were subcutaneous and were made as follow s :-

## R A B B I T S

| | RABBIT I | RABBIT II | RABBIT III | RABBIT IV | RABBIT V | RABBIT VI |
|---|---|---|---|---|---|---|
| DAY. OF INNOCULATION | INNOCULATED WITH "WHOLE" TYPH | INNOCULATED WITH "DETOXICATED" TYPHOID VACCINE | INNOCULATED WITH ALKALI SOL SUBS (from 5cc of Bacilli suspended in 6 cc SALINE | INNOCULATED WITH ACID SOL. SUBS. (from 6cc of Bacilli susp in 6 cc Saline. | INNOCULATED WITH ALCOHOL SOL SUBS (from 5cc. Bi susp in 6cc saline | INNOCULATED WITH PROTEOSES. Subs (from 5cc. Bacilli) |
| 1st " | 100 millions | 2.000 millions | 0·25 cc | 0·25 cc | 0·25 cc | 0·25 cc |
| 7th " | 100 " | 6,000 " | 0·50 cc | 0·50 cc | 0·50 cc | 0·50 cc |
| 13th " | 200 " | 10.000 " | 0·75 cc | 0·75 cc | 0·75 cc | 0·75 cc |
| 20th " | 500 " | 15,000 " | 1·00 cc | 1·00 cc | 1·00 cc | 1·00 cc |
| 27th " | 1000 " | 20,000 " | 1·50 cc | 1·50 cc | 1·50 cc | 1·50 cc |
| 34th " | 1000 " | 25,000 " | 2·00 cc | 2·00 cc | 2·00 cc | 2·00 cc |
| TOTAL | 2,900 " | 77,000 " | 6. 6cc contains .23 gms ALK sol | 6cc. contains ACID sol 0·1 gms SUBS. | .01 gms ALC. SOL SUBS | .04 gms. PROTEOSES. |

The rabbits immunised with the fractions each received the whole amount obtained from 5 cc of the semisolid mass of bacilli. To find out roughly how much this was, one cc of the semisolid mass of bacteria (centrifuged 1 hour at 3,000 revolutions per minute and weighing 1. 485 gms) was placed in a small crucible and dried in an oven at 50° C and then in a dessicator until the weight was constant. This was .098 gms. If the alkali soluble portion is about 60% of the bacillus 1 cc would correspond to .059 gms alkali soluble substance, but allowing as before for a loss of at least 20% during the process of detoxication .047 gms alkali soluble substance would correspond to 1 cc of whole bacilli. The rabbit receiving this fraction would be immunised with .23 gms alkali soluble substance.

After the first innoculation the rabbit receiving the acid soluble fraction became listless and refused food, and three days after the second injection, it died. A Post Mortem examination was performed but nothing abnormal was found. A second rabbit was then innoculated subcutaneously with the same suspension of acid soluble fraction as follows :-

Acid soluble fraction from 5 cc bacteria susupended in 6 cc saline.

| | | |
|---|---|---|
| 1st | day | . 1 cc |
| 4th | " | .15 cc |
| 7th | " | .25 cc |
| 10th | " | . 5 cc |
| 16th | " | .75 cc |
| 22nd | " | 1. 0 cc |
| 28th | " | 1. 0 cc |
| 34th | " | 1. 5 cc |
| TOTAL | | 6. 0 cc containing .1 gms acid soluble fraction. |

Of these rabbits (excluding the one which died) none showed any reaction clinically to their innoculations except the rabbit receiving the "whole" typhoid vaccine. It was listless and refused food for a day after the first dose was given, but it reacted less and less to the subsequent injections.

Some time later the rabbit which had received proteoses serum and the rabbit which had received whole typhoid serum, died. Both were examined Post Mortem and the findings showed pneumonia. At this time the heating of the animal house was faulty. New rabbits were immunised in exactly similar manner to replace these.

When it was seen that the findings given by the detoxicated typhoid serum were of a somewhat negative character, as shown in the following table of results, another rabbit and a fresh supply of vaccine were procured. This rabbit was innoculated intravenously as follows :-

| | |
|---|---|
| 1st day | 2,000 millions. |
| 2st day | 2,000 millions. |
| 2nd day | 2,000 millions. |
| 12th day | 5,000 millions. |
| 20th day | 10,000 millions. |
| 29th day | 20,000 millions. |
| 38th day | 30,000 millions. |
| TOTAL. | 71,000 millions. |

The sera of these rabbits were tested as before and their immunity estimated :-

1. By their ability to agglutinate Bacillus Typhosus.
2. " " " to act as bactericidal agents.
3. " " " to fix complement.

| Suspension | Serum from Normal Rabbit | "Whole" Typhoid Immune Serum | "Detoxicated" Typhoid Immune Serum. I | "Detoxicated" Typhoid Immune Serum II | Alkali Sol. Immune Serum | Acid Soluble Immune Serum | Alcohol Soluble Immune Serum | Proteoses Immune Serum |
|---|---|---|---|---|---|---|---|---|
| | 1/25 1/50 1/100 1/500 1/1000 | 1/25 1/50 1/100 1/500 1/1000 | 1/25 1/50 1/100 1/500 1/1000 | 1/25 1/50 1/100 1/500 1/1000 | 1/25 1/50 1/100 1/500 1/1000 | 1/25 1/50 1/100 1/500 1/1000 | 1/25 1/50 1/100 1/500 1/1000 | 1/25 1/50 1/100 1/500 1/1000 |
| 1. B. Typhosus vaccine ("Whole") | - - - - - | + + + + + | + + - - - | + + - - - | + + - - - | + + - - - | + - - - - | - - - - - |
| II Standard Emuls. "Dreyer" | - - - - - | + + + + + | + - - - - | + + - - - | + + + - - | + + - - - | + - - - - | - - - - - |
| | Precipitation not Tried Agglutination. | | | | | | | |
| "Detoxicated" Typhoid Vaccine (Genatosan) | - - - - - | ↓ ↓ ↓ ↓ ↓ - - | ↓ ↓ ↓ ↓ - - | ↓ ↓ ↓ - - - | ↓ ↓ ↓ - - | ↓ ↓ - - - | ↓ - - - - | - - - - - |
| Alkali Sol. Fraction. Suspended in Saline | - - - - - | ↓ ↓ ↓ - - | - - - - - | - - - - - | - ↓ ↓ - - | ↓ - - - - | - - - - - | - - - - - |
| | - - - - - | ↓ ↓ ↓ - - | - - - - - | - - - - - | ↓ - - - - | ↓ ↓ - - - | ↓ - - - - | - - - - - |
| Acid Sol Fraction. Suspended in Saline | - - - - - | ↓ ↓ ↓ - - | - - ↓ - - | - - - - - | - ↓ ↓ - - | ↓ - - - - | ↓ - - - - | - - - - - |
| | - - - - - | ↓ ↓ ↓ - - | - ↓ - - - | - - - - - | - ↓ ↓ - - | ↓ - - - - | - - - - - | - - - - - |
| Alcohol Sol Fraction Suspended in Saline | - - - - - | ↓ ↓ - - - | ↓ - - - - | ↓ - - - - | ↓ - - - - | ↓ ↓ - - - | ↓ - - - - | - - - - - |
| | - - - - - | ↓ ↓ - - - | - - - - - | - - - - - | ↓ ↓ - - - | ↓ - - - - | - - - - - | - - - - - |
| Proteoses Fraction Suspd in Saline | - - - - - | ↓ ↓ - - - | ↓ - - - - | - ↓ - - - | - - - - - | ↓ - - - - | - - - - - | - - - - - |
| | - - - - - | ↓ - - - - | ↓ - - - - | ↓ - - - - | - - - - - | ↓ - - - - | ↓ - - - - | - - - - - |

These agglutination tests were done by Dreyer's standard technique. The typhoid immune serum agglutinated a suspension of its own homologous organism up to 1 - 10,000 dilution et seq: and precipitated detoxicated typhoid vaccine in a dilution 1 - 1,000 and fraction suspensions in dilutions as follows; alkali suspension to 1 - 500, acid to 1 - 500, alcohol to 1 - 50, and proteoses to 1 - 50. The sera from the animals immunised with detoxicated typhoid (Genatosan) vaccine only agglutinated the emulsion of Bacillus Typhosus in case of Rabbit 1. to a dilution of 1 in 50. They formed a precipitate with detoxicated typhoid vaccine in a dilution of 1 in 1,000, but they formed no precipitate with a suspension of the alkali soluble fraction and they only precipitated the other "fraction" suspensions in low dilutions.

The alkali soluble immune serum agglutinated the emulsion of Bacillus Typhosus in a dilution of 1 in 50 and the standard emulsion of Bacillus Typhosus (Dreyer) in a dilution of 1 in 100. It formed precipitates with (1) detoxicated vaccine, (2) alkali soluble fraction, and (3) acid soluble fraction in dilutions of 1 in 100.

The acid soluble immune serum agglutinated the emulsion of Bacillus Typhosus in a dilution of 1 in 30 and the standard emulsion of Bacillus Typhosus (Dreyer) in the same dilution. It formed precipitates in dilutions of 1 in 25 with the other suspensions.

The serum from the rabbit immunised with the alcohol soluble substance only agglutinated Bacillus Typhosus and the standard emulsion in a dilution of 1 in 25 and it showed scarcely any precipitation with the other antigens.

The proteoses immune serum gave entirely negative results in all the tests.

Two patients attending the Venereal Clinic were innoculated with a total subcutaneous dosage of detoxicated typhoid vaccine (Genatosan) of 50,000 millions (3 doses. 10,000 ; 20,000 ; 20,000 millions at weekly intervals). They showed no clinical reaction beyond pain at the side of the innoculation after the first injection. There was no development of agglutinins to Bacillus Typhosus (Dreyer emulsion). One patient innoculated with 500 millions of "whole" Bacillus Typhosus vaccine developed agglutinins in dilutions up to 1 in 5,000.

From the results shown by these experiments, very slight agglutinative properties are developed in the sera of animals immunised with very large doses of the various detoxicated substances. The patients immunised with a total dosage of 50,000 millions developed no agglutinins whereas the patient immunised with 500 millions ($\frac{1}{10}$ of the former dosage) developed a serum with high agglutinative titre.

## COMPLEMENT FIXATION TESTS

| ANTIGEN | NORMAL RABBIT SERUM (COMPLEMENT) 2 | 3 | 4 | 5 | "WHOLE" TYPHOID IMM SERUM. (COMPLEMENT) 2 | 3 | 4 | 5 | "DETOXICATED" TYPHOID I IMMUNE SERUM 2 | 3 | 4 | 5 | "DETOXICATED" TYPHOID II IMM. SERUM 2 | 3 | 4 | 5 | ALKALI SOL FRACTION IMM. SERUM 2 | 3 | 4 | 5 | ACID SOL FRACTION IMM. SERUM 2 | 3 | 4 | 6 | ALCOHOL SOL FRACTION IMM. SERUM 2 | 3 | 4 | 5 | PROTEOSES FRACTION IMM. SERUM 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| "WHOLE" TYPHOID VACCINE | − | − | − | − | + | + | + | + | − | − | − | − | − | − | − | − | ± | − | − | − | + | − | ± | − | ± | − | − | − | ± | − | − | − | ± | − | − |
| | − | | − | | + | + | | | − | | − | | − | | − | | + | − | | | + | | ± | | ± | | − | | ± | | − | | ± | | |
| | − | | − | | + | + | | | − | | − | | − | | − | | + | − | | | + | | ± | | ± | | − | | ± | | − | | ± | | |
| "DETOXICATED" TYPHOID VACCINE | − | − | − | | ± | − | | | ± | ± | − | − | ± | ± | − | − | ± | − | − | | ± | − | − | | ± | − | − | | ± | − | − | | ± | − | − |
| | − | | − | | ± | − | | | ± | | − | | ± | | − | | + | − | | | ± | | − | | ± | | − | | ± | | − | | ± | | |
| | − | | − | | ± | − | | | ± | | − | | ± | | − | | ± | − | | | ± | | − | | ± | | − | | ± | | − | | ± | | |
| ALKALI SOL FRACTION (IN SALINE) | − | − | − | − | + | + | + | + | − | − | − | − | − | − | − | − | − | − | − | − | ± | − | − | − | ± | − | − | − | − | − | − | − | − | − | − |
| | − | | − | | + | | + | | − | | − | | − | | − | | ± | − | | | ± | | − | | ± | | − | | − | | − | | − | | |
| | − | | − | | + | | + | | − | | − | | − | | − | | ± | − | | | ± | | − | | ± | | − | | − | | − | | − | | |
| ACID SOL FRACTION (IN SALINE) | − | − | − | − | + | + | + | + | − | − | − | − | − | − | − | − | − | − | − | − | ± | ± | − | − | ± | − | − | − | − | − | − | − | − | − | − |
| | − | | − | | + | | + | | − | | − | | − | | − | | − | − | | | ± | | − | | ± | | − | | − | | − | | − | | |
| | − | | − | | + | | + | | − | | − | | − | | − | | − | − | | | ± | | − | | ± | | − | | − | | − | | − | | |
| ALCOHOL SOL FRACTION (IN SALINE) | − | − | − | | + | ± | | | − | − | − | | − | − | − | | ± | − | − | | ± | − | − | | + | − | − | | + | − | − | | + | ± | |
| | − | | − | | + | ± | | | − | | − | | − | | − | | ± | − | | | ± | | − | | + | | − | | ± | | − | | + | | |
| PROTEOSES FRACTION (IN SALINE) | − | − | − | − | + | + | ± | | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − |
| | − | | − | | + | + | | | − | | − | | − | | − | | − | − | | | − | | − | | − | | − | | − | | − | | ± | | |
| | − | | − | | + | + | | | − | | − | | − | | − | | − | − | | | − | | − | | − | | − | | − | | − | | ± | | |

These tests were done by three different methods.

(1) After Professor Campbell's (Capetown) method which is a modified No 4. (using 3 and 5 doses complement)

(2) No 4. method.

(3) After the method employed at Newcastle. i.e

The antigens used were :-

(1) An emulsion of 2 agar slopes of Bacillus Typhosus suspended in saline and heated at 60° C.

(2) Detoxicated typhoid vaccine (Genatosan) 20,000 millions per cc.

The total amounts of the fractions from 5 cc of a semisolid mass of bacilli suspended in 10 cc saline.

The typhoid immune serum in the presence of its homologous antigen held 3 doses of complement (it was not tried further) and with the "fraction" antigens it held 4 doses and 5 doses, while with detoxicated typhoid vaccine (Genatosan) it only showed faint fixation in the tube with 2 doses.

The sera from the detoxicated typhoid rabbits showed no ability to fix complement in the presence of a suspension of Bacillus Typhosus or suspensions of the fractions as antigens and showed faint fixation in the tube containing 2 doses, of complement in the presence of detoxicated Typhosus vaccine as antigen. The alkali soluble immune serum only held 3 doses of complement in the presence of Bacillus Typhosus emulsion as antigen, and it barely fixed 2 doses with the detoxicated Bacillus Typhosus vaccine; alcohol soluble fraction suspension, and its own homologous fraction as antigens. There was no fixation of complement in the presence of the acid soluble fraction as antigen. The acid soluble immune serum fixed three doses complement with Bacillus Typhosus emulsion as antigen, but it showed only partial fixation of 2 doses in the presence of the detoxicated typhoid vaccine and the alkali and acid soluble fractions as antigens. With proteoses as antigen there was no fixation. The alcohol soluble and the proteoses immune sera gave practically negative results,

Although these "fraction" immune sera appear to give slightly greater fixation with the suspension of "whole" Bacillus Typhosus than with their own homologous fractions the increase is too small to warrant a definite conclusion. The four fractions themselves made good antigens and with "whole" typhoid immune serum were capable of fixing four doses of complement.

Thomson found that the proteoses fraction of the Gonococcus was antigenic, but the proteoses fraction of Bacillus Typhosus as estimated by the agglutination and complement fixation tests above showed no antigenic properties.

The sera of the two patients who had been immunised with detoxicated typhoid vaccine showed no ability to fix complement in the presence of Bacillus Typhosus as antigen.

immune serum fixed three doses complement with Bacillus Typhosus emulsion as antigen, but it showed only partial fixation of 2 doses in the presence of the detoxicated typhoid vaccine and the alkali and acid soluble fractions as antigens. With proteoses as antigen there was no fixation. The alcohol soluble and the proteoses immune sera gave practically negative results.

Although these "fraction" immune sera appear to give slightly greater fixation with the suspension of "whole" Bacillus Typhosus than with their own homologous fractions the increase is too small to warrant a definite conclusion. The four fractions themselves made good antigens and with "whole" typhoid immune serum were capable of deviating four doses of complement.

Thomson found that the proteoses fraction of the Gonococcus was antigenic, but the proteoses fraction of Bacillus Typhosus as estimated by the agglutination and complement fixation tests above, showed no antigenic properties.

The sera of the two patients who had been immunised with detoxicated typhoid vaccine showed no ability to fix complement in the presence of Bacillus Typhosus as antigen.

Whereas the serum of the patient immunised with "whole" Bacillus Typhosus fixed 5 doses of complement.

Since this work was undertaken Thomson has reverted to the methods of detoxication as adopted by him in 1919. i.e. by dissolving the bacilli in weak alkali after they have been thoroughly broken up by a smashing machine.

He has discarded the treatment with alcohol because alcohol tends to reduce the antigenic value of the fraction very appreciably.

The following investigation was then undertaken to see whether treatment with a weak alkali had any detrimental effect on the germ.

One cc of a semisolid mass of Bacillus Typhosus was put into each of three tubes. To the first 10 cc of distilled water were added and the tube was sealed with a rubber cork and wax. To the second tube 10 cc of saline were added and the tube was sealed in like manner. To the third tube 10 cc of N/20 NaOH were added and this tube was also sealed. The tubes were then put into the 37. 5° C incubator and left there. They were shaken vigorously at times. At the end of six weeks they were removed and the following observations were made. The supernatant bacteria-free fluids from tubes 1 and 2 used as antigens with typhoid immune serum held six doses of complement, while the bacterial deposits from these tubes (after being washed quickly in saline) held only two doses of complement under similar conditions. The supernatant bacteria-free fluid from tube three neutralised and used as an antigen with typhoid immune serum held 2 doses of complement; the bacterial deposit from this tube subjected to similar treatment also held two doses of complement. Ferry and Fisher find that treating germs with $\frac{N}{10}$ NaOH reduces their antigenic powers as tested by the complement fixation and agglutination tests. They do not state how long the germs were in contact with the NaOH.[13]

The following tests for the bactericidal properties of immune the sera were performed in vitro by Stern and Kortes method. This method was used because the rabbits had to be destroyed owing to an outbreak of disease in the animal house. (Post Mortem findings showed no abnormalities).

## Doses of Sera used.

| | 1cc serum | .01 serum | .002 cc serum | .001 cc serum | .0001 cc serum |
|---|---|---|---|---|---|
| "WHOLE" Bacillus Typhosus Serum | + + + | + + | practically sterile | very few | + + |
| "DETOXICATED" Typhoid vaccine | + + + + | + + + + | + + | + + | + + + + |
| GENALOSAN IMMUNE SERUM | + + + + | + + + + | + + + | + + + | + + + + |
| ALKALI Soluble FRACTION IMMUNE SERUM | + + + + | + + + + | + + + | + + + | + + + + |
| Acid Soluble FRACTION IMMUNE SERUM | + + + + | + + + + | + + | + + + | + + + + |

In this experiment all the sera showed the so-called"deflection of complement"which was probably due to the fact that they were more than a week old when the experiment was performed. Only the typhoid immune serum showed bactericidal properties in dilutions of .002 and .001 of neat serum. There was a certain deduction in the number of colonies on the plates with the similar doses of the other immune sera, but even so, the number of each plate was too large to count. It would seem from these results that the sera from animals immunised with the various detoxicated substances have few if any bactericidal properties.

An investigation has been carried out into the effect of detoxication by Thomson's methods on the antigenic properties of Bacillus Typhosus.

It has been found that the antigenic properties as shown by the usual methods viz. the agglutination, the precipitation, bactericidal and complement fixation tests of a suspension of "Whole" Bacillus Typhosus are infinitely greater than either the whole(detoxicated ) Bacillus Typhosus vaccine or any of its fractions.

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